REVIEW

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The endometrial regeneration frontiers: from mechanisms to applications in regenerative medicine

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Abstract

The endometrium is a unique and remarkable tissue characterized by a constant regeneration activity and this has been speculative for scientists, regarding its mechanism, regulatory factors, and their significance for fertility and endometrial pathology. Relatively recent scientific progresses due to genomics, proteomics, and transcriptomics have changed the knowledge in respect with endometrial regeneration and uterine-derived diseases. Our review is designed to highlight the recent progresses in understanding the endometrial physiology and its alterations involvement in uterine-derived diseases, addressing the current paradigm regarding endometrial regeneration, based on endometrial regenerative cells. In an attempt to explain the complex process of endometrial regeneration, different mechanisms have been proposed during time, from proliferation of basal glandular cells, to mesenchymal–epithelial transition, and lately to differentiation of stromal cells, based on endometrial regenerative cells or stem cells. Their unlimited potential of reconstruction of any type of tissue has been demonstrated and is currently in different trial stages in cell-based therapies of regenerative medicine, opening promising perspectives in severe or lethal diseases, by exploitation of stem cells. Currently, beside uterine acquired diseases and infertility, endometrial stem cells have been tested in a large spectrum of clinical applications. The great potential of endometrial cells for cell therapies arise from their accessibility, completely xeno-free derivation, allogenic use, possibility of large-scale therapeutic doses production, safety, reproducibility, and chance to overcome the drawbacks associated with autologous therapies. In order to overcome hostile environment of an injured tissue, the association of endometrial stem cells with other cells or added medium opens the perspective of specific combination available as standardized therapeutic means in the next future.

Keywords: endometrial regeneration, stem cells, endometrial regenerative cells, mesenchymal–epithelial transition, regenerative medicine.

Introduction

The uterus is a particular organ characterized by its morphological adaptation to the reproduction function. Both myometrium and endometrium are able to modify their histological structure to support the embryo development. Its implantation and nourishment is possible due to dramatic changes of endometrium and its protection and delivery is achieved by myometrium. The cyclical evolution of endometrium is governed by ovarian steroid hormones, under the modulation of neuroendocrine hypothalamo–hypophyseal system.

Considering these important functions, the endometrium is a unique and remarkable tissue characterized by a regeneration activity comparable to that of bone marrow, epidermis, and intestinal epithelium. In humans, it undergoes 400–500 menstrual cycles during a woman's reproductive lifetime and this high turnover has been speculative for scientists, regarding its mechanism, regulatory factors, and their significance for fertility and endometrial pathology [1, 2]. Relatively recent scientific progresses due to genomics, proteomics, and transcriptomics have changed the knowledge in respect with endometrial regeneration and uterine-derived diseases. The latter seem to be based on unbalanced factors involved in proliferation regulation, being lately reflected in several *World Health Organization* (WHO) classifications changes, which have been traditionally based on clinicopathological features.

Our review is designed to highlight the recent progresses in understanding the endometrial physiology and its alterations involvement in uterine-derived diseases, addressing the current paradigm regarding endometrial regeneration, based on endometrial regenerative cells (ERCs). Their unlimited potential of reconstruction of any type of tissue has been demonstrated and is currently in different trial stages in cell-based therapies opening promising perspectives in severe or lethal diseases. A revolutionary therapeutic approach type of medicine has emerged by exploitation of stem cells, namely regenerative medicine, and due to their unique characteristics, the exploitation of ERCs has revealed significant advantages compared to other types of stem cells. The paper is based on English language publications indexed in the main medical databases, using the following searching keywords: "endometrium", "stem cells", "regeneration", "mesenchymal–epithelial transition", and "regenerative medicine".

Past theories of endometrial regeneration mechanism

Based on the endometrial zonation of primates [3], there are several compartments, according to their glandular

or epithelial content, as following: two basal layers (IV: base and III: middle of endometrial glands) and the two functional layers (II: upper endometrial glands and I: luminal epithelium) which have been later characterized by marked proliferation kinetics differences [4] (Figure 1).

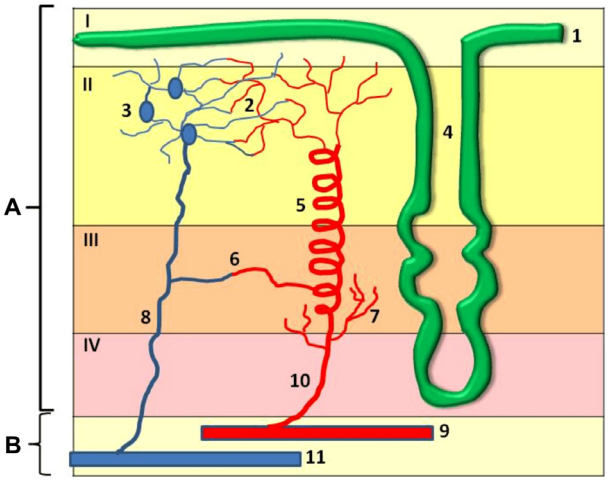

Figure 1 – ***The endometrial zonation model. Based on the endometrial zonation of primates, there are four compartments: basal layer IV, containing the glandular bases, basal layer III, with the middle of the endometrial glands, functional layer II, containing the upper endometrial glands, and functional layer I corresponding to the luminal epithelium. A: Endometrium; B: Myometrium; I: Luminal epithelium; II: Upper endometrial glands; III: Middle of endometrial glands; IV: Basal endometrial glands; 1: Lining endometrial epithelium; 2: Capillary plexus; 3: Venous lake; 4: Endometrial gland; 5: Spiral artery; 6: Arteriovenous anastomosis; 7: Straight artery; 8: Vein; 9: Arcuate artery; 10: Radial branch/artery; 11: Arcuate vein.***

In an attempt to explain the complex process of endometrial regeneration, two past mechanisms have been proposed during time, as following:

The first mechanism has been initially elaborated several decades ago, based on the capacity of glandular epithelial cells to proliferate. It had been hypothesized that primate and human endometria are regenerating by *epithelial cell proliferation of the upper ends of the gland stumps from basal layer* [5]. The endometrial regeneration has been considered a unique process in which basal layer acts as a germinal layer due to its particular vascularization and different hormonal influences when compared to the functional layer shed with each menstruation. The speculation that the "free-edge" effect initiates endometrial re-epithelialization has been launched considering that the lack of endometrial neighboring cells in the denuded area may elicit a growth signal [6].

Observing the capacity of remnant stromal cells to act under hormonal stimulus and regenerate the surface endometrium, the *hypothesis of mesenchymal–epithelial transition* has been proposed as an alternative mechanism. The mechanism of conversion from epithelial to mesenchymal phenotype is used in morphogenesis, in tissue repair [7], and is also reinitiated during cancer invasion [8]. During this stepwise process, epithelial cells lose cell–cell adhesion molecules, the apical–basal polarity and achieve a more stromal-type histological phenotype [7]. This is characterized by down-regulation of epithelial markers, such as E-cadherin, α- and β-catenins, cytokeratins, and claudin, and acquisition of mesenchymal markers, such as N-cadherin, cadherin-11, along with S100A4, vimentin, fibronectin, α-smooth muscle actin (α-SMA), matrix metalloproteinases (MMPs) (MMP-2, MMP-3, and MMP-9) expression, under the control of transcription factors like TWIST, SNAIL, SLUG, and ZEB1 [8, 9]. Conversely, the reverse process is represented by the mesenchymal–epithelial transition (MET), as a necessary step in morphogenesis, to continue some differentiation pathways [7], and seems to be reinitiated in tumor metastases. Therefore, malignant cells undergo epithelial–mesenchymal transition (EMT) to invade and disseminate and then undergo a reverse process (MET) to form epithelial metastases in target organs [8].

The first evidences of the involvement of stromal cells in endometrial regeneration have been provided by electron microscopy [10, 11] and later on by murine models [12]. Thus, stromal cells are considered to be programmed to lose their mesenchymal traits and gain epithelial features, as demonstrated by co-expression of pan-cytokeratin (pan-CK) with vimentin, after 24 hours of progesterone withdrawal [12], expression of genes involved in MET, and identification of proliferative activity in both stromal and epithelial areas [13].

Furthermore, an important contribution of stromal cells from the functional layer in regeneration of extracellular matrix has been demonstrated by laser capture microdissection technique [14].

In view of an analogy with a wound healing process, a regulated balance between MET and the reverse, EMT should be active in endometrium, in order to prevent extreme proliferation of apoptosis resistant myofibroblasts, with possible excessive production of type I collagen leading to fibrosis [13].

The endometrial regulation of the balance between these "mirror" processes is attributed to hypoxia, components of the extracellular matrix, cytokines, and growth factors [15], without an associated scarring process. Not surprisingly, stromal cells from endometriotic implants express higher levels of α-SMA when compared to stromal endometrial cells, leading to the hypothesis that alteration in the regulation of this process may result in endometriosis [13].

Recent theory of endometrial regeneration mechanism

The initial hypothesis of epithelial glandular stumps proliferation [5] has been later questioned [16] and further on invalidated by complex hysteroscopical–histological–electron microscopic studies that demonstrated the regeneration of surface endometrium by *differentiation of stromal cells* [11].

Supplementary, more and more evidences of stem cells location in endometrium, analogous to other organs counterparts, have been supporting the hypothesis of possible reconstruction of endometrium based on *endometrial regenerative cells or stem cells*.

The existence of human endometrial stem cells has been initially hypothesized by Prianishnikov, in 1978, who identified three types of endometrial proliferative cells, according to their correlation with steroids hormones, as

follows: estradiol-sensitive, progesterone-sensitive, and estradiol- and progesterone-sensitive cells [17]. Studies of endometrial derived colony-forming units have been later added to support this idea [18]. Moreover, murine models have been very useful in identifying stem cells, by 5-bromo-2'-deoxyuridine (BrdU) labeling and proliferating cell nuclear antigen (PCNA) immunofluorescence [19].

The identification of predecidual cells sharing bone marrow derived features, in 1982 [20], together with the expression of telomerase gene [21], along with c-kit and OCT-4 (markers of stem/puripotential cells) [22] lead to the hypothesis of stem-like endometrial cells. Later on, clonogenicity studies have identified cells capable of stromal and epithelial generation [23, 24].

Based on the latest findings, a recent model of both ectopic and eutopic implantation has been proposed [25], as an alternative mechanism of functional layer regeneration, based on basalis persistence during menstruation. This model involves stem/progenitor cells, possible bone marrow-derived, located in the vascular or perivascular areas, in both basal and functional layers and contained in the sloughed endometrium. They may either implant in ectopic sites, by retrograde menstruation, either remain inside the uterine cavity after menstruation and later on these *stem cells reimplant in regenerating endometrium*. The latter event is also supported by previous observations of heterogeneous areas regarding their development stage, noticed in normal endometrium [11].

The endothelial location of stem cells is in agreement with the speculations about their bone marrow origin and their ability to participate in neovascularization and neoendothelialization [26] and, furthermore, to epithelial and stromal endometrial cells regeneration [27]. Moreover, circulating stem cells, mainly introduced after surgical trauma or due to increased vascular turbulence, may result in lymphovascular dissemination, followed by ectopic implantation [28] (Figure 2).

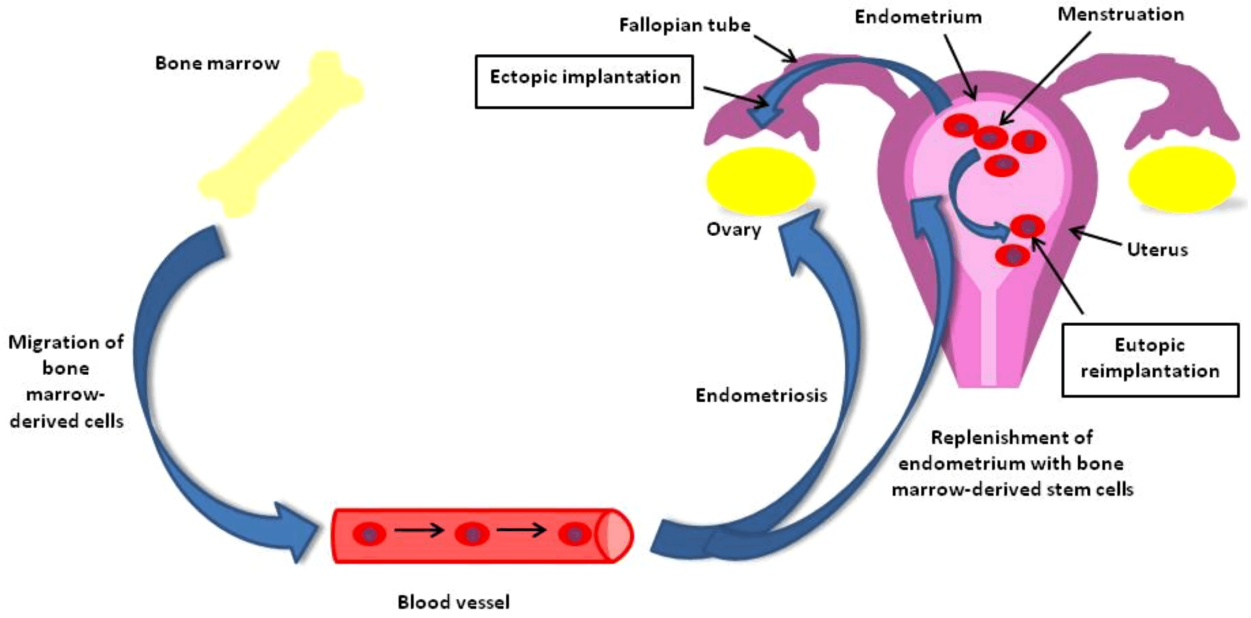

Figure 2 – ***Current model of endometrial regeneration. The recent model of both ectopic and eutopic implantation is based on stem/progenitor cells, possible bone marrow-derived, located in both basal and functional layers and also contained in the sloughed endometrium; endometrial stem cells may either implant in ectopic sites, by retrograde menstruation, either remain inside the uterine cavity and later on they reimplant and regenerate the endometrium; circulating stem cells may also result in lymphovascular dissemination, followed by ectopic implantation.***

Angiogenesis and MMPs role in endometrium regeneration

The processes involved in endometrial repair, analogous to classic wound healing, are beginning during the first 24 hours after the initiation of tissue fragmentation, as a mechanism of tissue damage minimization, comprising inflammation and its resolution, angiogenesis, tissue formation and remodeling [13].

On the background of menstrual endometrium, which appears torn, with glandular denuded surface, regeneration starts in menstrual day 2, in the absence of ovarian hormones influence [10, 29]. The process is completed until day 6 [13, 29], with alternation of shedding and repair areas forming a piecemeal pattern [11].

The completion of endometrial regeneration is estrogen-dependent, under mitogenic stimulation performed by a panel of cytokines and growth factors, such as transforming growth factor-alpha (TGF-α), epidermal growth factor (EGF) [both acting *via* epidermal growth factor receptor (EGFR)], platelet-derived growth factor (PDGF) [23], vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and basic fibroblast growth factor (bFGF) [30].

Another important feature contributing to endometrial regeneration is represented by endometrial vessels regrowth *via* a balanced angiogenesis or remodeling of the vascular network. The regulation of this process is attributed to complex interactions between VEGF family of proteins, FGFs, angiopoietins, angiogenin, ephrins, and their specific receptors [25].

The evidences from murine models support the idea of a later intervention of stromal response to steroids and consequent mitotic activity initiation, in the menstruation

days 5–6 [5]. There is a correlation between VEGF peak of expression in correlation with estrogen-dependent regeneration endometrial phase, identified in macaque models [13].

Another important player triggering VEGF stimulation is represented by progesterone withdrawal both in animal models and in human endometrial explants, with possible downstream mediators as hypoxia and prostaglandins (PGs) [31, 32].

The involvement of stromal cell-derived factor-1 (SDF-1) and its receptors, in endometrial vascular regeneration, has been demonstrated by *in vitro* studies, C-X-C chemokine receptor type 4 (CXCR4) [C-X-C motif chemokine ligand 12 (CXCL12) receptor] expression being strong both in epithelium and endothelium, in the early proliferative phase [33]. The hypothesis of possible autocrine endothelial signals in repairing process may partially support the particular mechanism of normal endometrial regeneration without scarring [13].

The balance between MMPs and tissue inhibitors of metalloproteinases (TIMPs) has a key role in endometrial remodeling, TIMP-1 and TIMP-2 exhibiting cell growth stimulatory ability, antiapoptotic capacity, and erythroid-potentiating activity [34]. Numerous studies have demonstrated MMPs involvement in a large spectrum of endometrial processes, such as apoptosis, proliferation, differentiation, and angiogenesis. This panel of activities is attributed to MMPs capacity to stimulate the hydrolysis of numerous substances which belong to variable categories, such as growth factors, cytokines precursors, proteinase inhibitors, hormone receptors, insulin-like growth factor-binding protein (IGFBP), interleukin-1beta (IL-1β), and serum amyloid A [34]. Therefore, uterine reshufflings seem to be the result of the complex activities of MMPs and their specific inhibitors.

MMPs register a large variability of expression corresponding to different phases of the endometrial cycle. For instance, in the proliferative phase, a strong MMP-7 epithelial expression has been registered [34]. This is associated to a strong MMP-9 expression in arterioles, which are developing within the supporting stroma [34]. Furthermore, endometrial stroma has an enhanced expression of other MMPs, such as membrane type-1 (MT1)-MMP, along with MMP-11, MMP-10, MMP-3, MMP-2, and MMP-1 [34].

While MMP-7 epithelial expression is also consistent with the secretory phase, other MMPs become co-expressed in advanced secretory stage, such as MMP-11 and MMP-10 [34]. An interesting strong MMP-2 expression is noticed in vascular and stromal tissue in secretory phase, suggesting its correlation with the process of angiogenesis [34].

Another close correlation has been demonstrated between MMP-2 strong stromal expression and VEGF expression in endometrium [34]. This co-expression is stimulated by estradiol and, possibly, by the contribution of concomitant hypoxia [34]. Moreover, newly formed capillaries of proliferating endometrium co-express MMPs and VEGF [30]. Vascular smooth muscle cells register an enhanced MMPs expression as a result of stromal and epithelial cells secretion of several proinflammatory cytokines [30].

Types of adult stem cells

Adult, somatic, or tissue-specific stem cells are dispersed throughout the whole body, in bone marrow [35], cord blood [36], Wharton's jelly [37], dental pulp [38], peripheral blood [39], and Fallopian tube [40]. Longtime after the embryonic development, these cells maintain their undifferentiated state, their capacity of self-renewal, by their capacity to generate identical daughter cells. These are resting in quiescent functional status, being able of multi-lineage differentiation, by asymmetrical divisions, and transformation into committed cells that may reconstitute the tissue where they reside. Due to their role in replenishment and regeneration of damaged or dead tissues, they possess the capacity of morphological and functional tissue maintenance. This unique characteristic led to the idea that they have the ability to regenerate the entire organ where they are located [41].

A viable solution in the treatment of numerous degenerative diseases is represented by stem cells therapies and thus clinical trials are currently trying to assess their compliance for human application. Due to ethical controversies and to the risk of tumorigenesis, the practical exploitation of stem cells has been delayed. By elimination of the main drawbacks, adult mesenchymal stem cells (MSCs), firstly identified in bone marrow, and later on in periosteum, skeletal muscle, pancreas, placenta [42], adipose tissue [43], and dental pulp [44] emerged as a viable solution in both heterologous and autologous cell transplant [2].

MSCs are defined by the *Committee of the International Society for Cellular Therapy* as plastic adherent multipotent cells, able to differentiate *in vitro* into adipocytes, chondroblasts, and osteoblasts ("orthodox pathway"), with positive expression of CD73, CD90, and CD105, and negative expression of human leukocyte antigen–antigen D related (HLA-DR), CD11b, CD14, CD19, CD34, CD45, and CD79a [45]. Their potential of differentiation into osteocytes and chondrocytes had been already exploited in bone and cartilage repair [46].

Beside the "orthodox" differentiation, a "non-orthodox pathway" has been also demonstrated, towards muscle [47], neurons [48], pancreatic islets [49], and hepatocytes [50].

The study of bone marrow-derived MSCs (BM-MSCs) has demonstrated their role in the inhibition of immune system, by suppressing T-cells proliferation and diverting their differentiation into tolerogenic T-regulatory cells instead of proinflammatory Th-cells [51]. Supplementary, BM-MSCs suppress natural killer (NK) cells, switch macrophages phenotype from type 1 to type 2 (pro-inflammatory to anti-immunomodulatory) [51], induce a dendritic cells tolerogenic phenotype [52], secrete chemokines for MSCs recruitment [C–C motif chemokine ligand 2 (CCL2), CXCL8, and CXCL12], and a variety of cytokines and growth factors with antiapoptotic [TGF-β, bFGF, IGF-1, and hepatocyte growth factor (HGF)], angiogenic [VEGF, bFGF, phosphatidylinositol-glycan biosynthesis class F protein (PIGF), and monocyte chemoattractant protein-1 (MCP-1)], and supportive functions [macrophage colony-stimulating factor (M-CSF), IL6, and SDF-1] [2]. Their ability of immunomodulation had been already demonstrated both *in vitro* and *in vivo*,

creating the premises for their use in both autologous and heterologous applications [53].

Endometrial regenerative cells (ERCs) identification

Since 1978, the existence of stem cells in the endometrium has been speculated, firstly as estradiol-sensitive, progesterone-sensitive, and estradiol-progesterone-sensitive cells [17], then correlated to a bone marrow origin [20], and later supported by telomerase expression in proliferative endometrium [21].

Within this context, the endometrium has been revealed as a source of stem cells useful in therapy, according to two independent research teams [54, 55]. The first group used cells derived from menstrual blood followed by cloning in order to obtain a pluripotent population, named ERCs [54]. The second group used c-kit selection of mononuclears from the menstrual blood that have also showed a marked proliferative ability [55].

The identification of endometrial pluripotent stem cells from menstrual blood, generated a population showing telomerase+, octamer-binding transcription factor 4 (OCT4)+, CD9+, CD29+, CD41a+, CD44+, CD59+, CD73+, CD90+, CD105+, MSI1+, NOTCH1+ [56–58], along with CD34+ and CD117+, in the basal layer [59], and CD133+ in the epithelial component [57], while other markers showed lack of expression (NANOG-1-, STRO1-, CD14-, and CD45-) [54]). These cells exhibited the ability to differentiate *in vitro* into 11 different lineages: endothelial, respiratory epithelium, adipocytic [60], chondrogenic [56, 61], osteogenic [56, 62], myocytic [63], neural [64], hepatic, pancreatic lineages, oligodendrocytic [62], and odontoblastic [65] and have been named "endometrial regenerative cells" (ERCs) [54] (Figure 3).

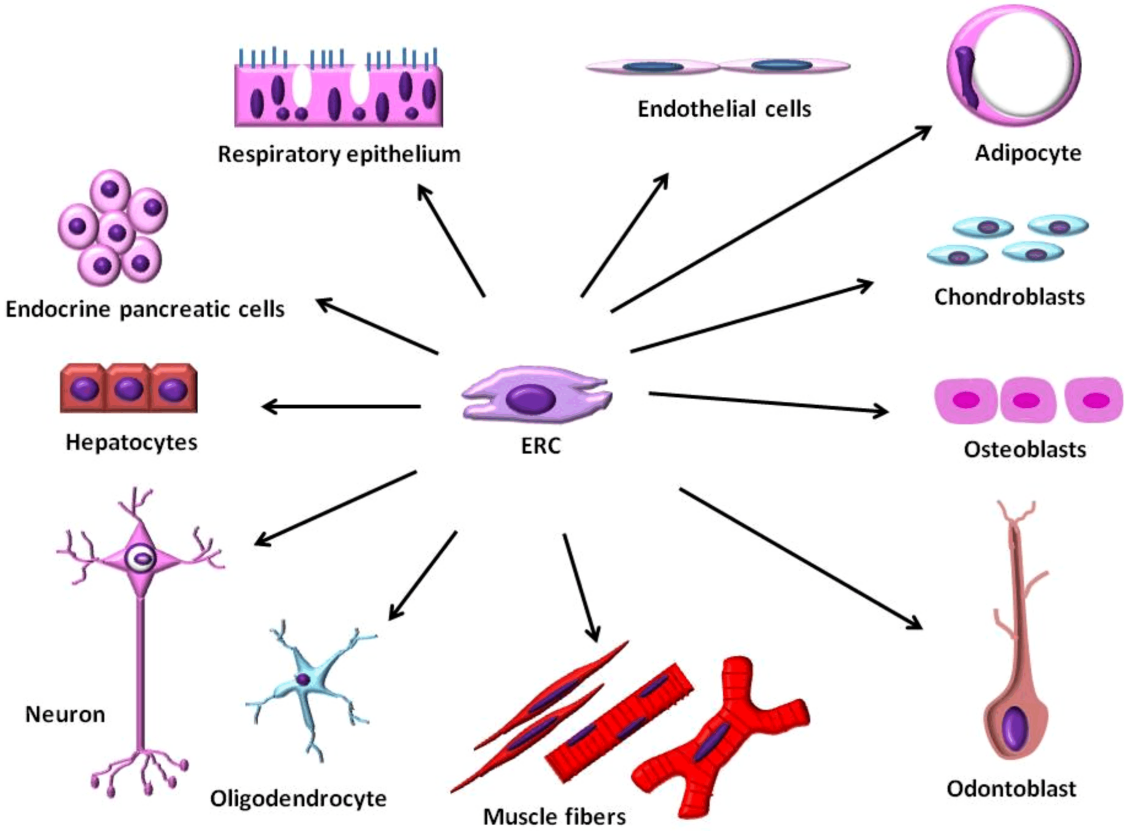

Figure 3 – ***Multipotentiality of endometrial regenerative cells (ERCs). Endometrial regenerative cells (ERCs) have demonstrated*** **in vitro** ***ability to differentiate into eleven different types of cells.***

Using menstrual blood mononuclear cells c-kit selection, similar cells have been identified [55]. During ERCs reprogramming, endogenous NANOG becomes expressed [66].

ERCs have a high proliferative activity, with ≥30 doublings [18, 67] and display important role in angiogenesis, as demonstrated in a hind limb ischemia model [68].

Putative endometrial stem cells located in the basalis have been firstly suggested decades ago [17] and much later the demonstration of monoclonality added a convincing support to this supposition [69]. In humans, endometrial stem cells have been identified by their ability to form colonies in cultures [23]. While stromal cells exhibited an increased *in vivo* capacity, with a peak clonogenicity in proliferative phase, their epithelial counterparts, later on identified, demonstrated their highest activity in secretory phase [24].

Their characterization has been further performed [27, 67], with both stromal and epithelial cells exhibiting clonogenicity. The endometrial stem cells have been described as fibroblast-like cells, with adherence to plastic ability and multipotentiality *in vitro* [56].

In terms of the required growth factors, in serum-free medium, two types of clones may be obtained [23, 24], considered to belong to different endometrial niches (epithelial *vs.* stromal), as illustrated in Figure 4.

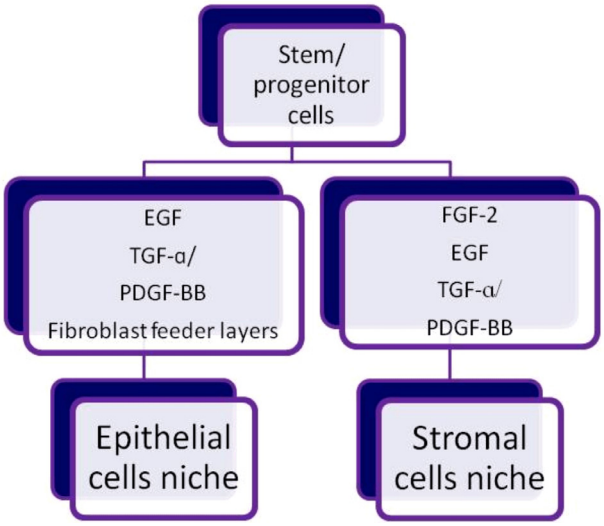

Figure 4 – ***Clonogenic development of epithelial or stromal endometrial cells. Different types of medium are necessary for the clonogenic development of either epithelial or stromal endometrial cells (EGF, TGF-α/PDGF-BB, on fibroblast feeder layers* vs. *FGF-2, EGF, TGF-α/PDGF-BB). EGF: Epidermal growth factor; FGF-2: Basic fibroblast growth factor (bFGF); TGF-α/PDGF-BB: Transforming growth factor-alpha/Platelet-derived growth factor two B subunits.***

Endometrial pluripotent cells may be associated with endometrial angiogenesis [1], considering their co-expression of MMPs and angiogenic factors [54]. It is already recognized the supporting role of estradiol in VEGF production and, consequently, in endometrial vasculogenesis [54, 55, 70]. Beside NK cells [71], neutrophils [72], and circulating endothelial progenitor cells [73], endometrial pluripotent cells appear to play a pivotal role in the multifactorial process of angiogenesis [1].

More and more evidences are confirming the hypothesis that adult stem cells are also present in mouse female reproductive tracts [25, 67]. Although a plethora of experimental and *in vitro* models have been tested, due to major differences between rodents and humans and poor reflection of the steps involved in the endometrial regeneration, the progresses in understanding the phenomena has been delayed. However, there are numerous evidences of adult stem cells located in human and mouse female reproductive tracts [25, 67].

Several teams of research have identified endometrial stem cells not only in the basal layer but also in the functional layer, being associated with the endothelium [74, 75]. *In vivo* animal models developed to study endometriosis (ectopic functional endometrial-like tissue) provided useful information regarding the endometrial regeneration. The experiments using transplanted human endometrial cells under the renal capsule of immunodeficient NOG mice demonstrated the existence of human endothelial cells/progenitors able to form a chimeric vascular system [26].

Moreover, using the study of epigenetic changes, by analyzing the methylation patterns, a diversity of stem cells has been also identified in aging atrophic endometrium demonstrating their persistence throughout entire life, in correlation with clinical data of the regenerative capacities elicited by hormonal replacement therapies [24].

ERCs possess a larger spectrum of potentiality than expected and thus its potential use in regenerative medicine seems very extensive. Furthermore, new molecular techniques [enzyme-linked immunosorbent assay (ELISA), reverse transcription–polymerase chain reaction (RT-PCR), high performance liquid chromatography (HPLC) quantification, flow cytometry, ribonucleic acid (RNA) and protein microarray, fluorescence-activated cell sorting (FACS), and whole transcriptome shotgun sequencing (WTSS)] may identify the trophic substances involved in the regulation of this process and thus, may reveal their potential utility in stimulation of stem cells activity [2, 76].

However, the specific conditions required for the niche to exhibit its stem cell activity are difficult to reproduce in different *in vitro* and *in vivo* experiments, mainly because the host cell population also contains precursors/progenitors [25, 67].

Interestingly, one of the sources of endometrial stem cells is also the bone marrow, contributing to the cellular turnover and being able to react to inflammatory stimuli [27], as reflected into endometrial glandular chimerism.

Side population and transit amplifying cells

Stem subpopulation or "side population" (SP) cells isolated in mammals are associated with adenosine triphosphate (ATP-binding cassette transporter protein (ABCG2/Bcrp1) and show multilineage development capacities [77].

SP cells are round, small, have self-renewal abilities, a long lifespan and proliferation activity, acting as progenitor cells [77]. SP are negative for CD9 (endometrial glandular marker) and CD13 (endometrial stromal marker), are able to extend podia, and may be maintained in cultures up to nine months [77].

The enhanced tumorigenicity of SP cells, together with the bipotent epithelial and stromal population generation, along with enhanced migration ability might be linked to EMT [77].

An important recent finding was that of constant identification of endometrial MSCs CD140b+/PDGFRβ and CD146+ not only in basal, but also, in a lesser extent, in functional endometrium, showing the maximum capacity of self-renewal in menstrual phase but also continued in proliferative phase [78]. Therefore, these cells are those responsible for endometrial regeneration, representing approximate 1.5% of endometrial stromal cells (quiescent and activated stem cells) [78].

The identification of stem cells in functionalis zone supports the correlation with endometriosis, as basal layer is not involved in menstruation [78] and furthermore endometrial MSCs inability to decidualize, due to a progesterone resistance, may be attributed to a stem cell disease [78].

Moreover, it has been hypothesized that stem/progenitor cells from basal zone may form large colony-forming units (CFUs), while transit amplifying cells from functionalis zone, more differentiated, form small CFUs [79]. At the endometrial–myometrial junction and in perivascular locations a CD146+ PDGFRβ MSC population has been identified [56, 80], exhibiting multilineage abilities. Thus, a common origin with bone marrow-derived human mesenchymal has been suggested [18].

Control factors of ERCs

The stimulating factors of endometrial stem cells are the following growth factors: PDGFRβ, EGF, TGF-α, and bFGF [80].

As the differentiated stromal cells originating in stem cells express estrogen receptor beta (ERβ), progesterone receptor (PR), luteinizing hormone (LH), follicle-stimulating hormone (FSH), they may develop decidual markers under the stimulation of progesterone (P4), estradiol (E2), androstenedione, bone morphogenetic protein 2 (BMP2), and activators of the protein kinase A (PKA) pathway, confirming the intimate relationship with bone marrow-derived human mesenchymal cells [79].

Among the stromal cells regulators, a network of regulators, such as BMP7, wingless (Wnt)/β-catenin, forkhead box protein O1A (FOXOA1), CCAAT/enhancer-binding protein beta (C/EBPβ), homeobox A10 (HOXA10), Dickkopf-1 (Dkk1), Wnt, and death effector domain-containing protein (DEDD) have been identified [79].

In vitro and *in vivo* endometrial models

The unique capacities of regeneration have been exploited in experimental models as attempts of endometrial reconstruction [81]. *In vivo* attempts have used endometrial cells [82], associated to SP cells in renal subcapsular transplantation [26, 75], or associated to blastocysts, as three-dimensional models of implantation [83].

In vitro models have used endometrial cells embedded in a variety of extracellular matrix components [84], matrigel [85], or collagen-basement membrane matrix [86].

Other models have been inspired by the normal uterine organization, and therefore have used smooth muscle in collagen–matrigel system, as a base for endometrial cells [87].

Moreover, an *in vitro* model using human endometrial CD146+ stem cells cultured in a collagen–matrigel (set of macromolecules, such as collagen, laminin, entactin, and growth factors) scaffold containing uterine smooth muscle cells has been successfully achieved [62].

It seems that the underlying smooth muscle is inducing epithelial endometrial tissue, by secretion of ghrelin, nidogen-1 and nidogen-2, and is mandatory for glandular structures appearance [62]. The switch from CD146+ to CD9+, along with laminin subunit alpha 2 (LAMA2), collagen alpha-1(IV) chain (COL4A1), zonula occludens-1 (ZO-1), MMP-2, and secreted phosphoprotein 1 (SPP1) expression, are suggestive for the involvement of MET in this process [62].

Endometrial cancer stem cells

Recent research has identified a particular type of stem cells which have been considered as responsible of invasion, metastasis, and the development of resistance to conventional therapy, called cancer stem cells (CSCs) [88], having its counterpart in endometrium.

Moreover, tumor milieu has reciprocal interactions with malignant cells. Therefore, stromal matrix is inducing CSCs proliferation, while a specific epithelial cells phenotype induces EMT, followed by invasion, metastasis, along with hormonal, chemo-, and radiotherapy resistance acquisition in different tumors, including endometrial carcinoma [88].

Several markers have emerged as useful for identification of CSCs, such as human prominin-1 (CD133), CD44, Nanog1, Sall4 [88], along with CXCR4, c-Myc, Sox-2, Oct4A, ATP-binding cassette subfamily G member 2 (ABCG2), BMI-1, CK18, nestin, β-actin [89], and telomerase [90].

CD133 represents a member of the prominin family, a membrane glycoprotein, which is associated with poor prognosis in endometrial endometrioid carcinoma [88].

A known adhesion molecule, CD44, is another CSC marker, and its expression seems to be correlated with a higher aggressiveness of endometrioid endometrial carcinoma [88]). As a consequence, both CD133 and CD44 may be associated with carcinoma progression and poor prognosis [88].

Nanog, Oct4, and Sox-1 may activate their own genes, resulting in self-renewal abilities [89].

Sall4 [91] is a member of the spalt-like (SALL) gene family, which is responsible for the persistence of self-renewal and pluripotent capacities of embryonic stem cells (ESCs). While SALL4 is registering a progressive loss in expression after birth, being absent in most adult tissues, it becomes re-expressed during carcinogenesis [92, 93]. Therefore, it has been identified in different cancers, including endometrial cancer, being associated to their ability to metastasize and to develop drug resistance [92, 93].

CXCR4 represents a stromal cell-derived factor-1 receptor and its stimulation results in several tumor characteristics, which increase its aggressive behavior [89].

ABCG2 is a marker of a fraction of SP cells, containing ATP-binding cassette transporter G2 that result in the capacity to pump out intracellular deoxyribonucleic acid (DNA)-binding dye Hoechst 33342 [25].

BMI-1 belongs to polycomb genes and is associated with the self-renewal capacity [94].

CK18 has been identified as an independent factor associated with poor prognosis in some cancers [95].

Nestin is an intermediate filament protein identified as a stem cell marker in endometrial cancer [96].

However, if the markers of EMT are permanently expressed, a correlation with the development of carcinosarcomas has been demonstrated [88].

Thus, the downregulation of hormone receptors may be significant for invasion and metastasis and, added to the expression of CSCs markers and loss of E-cadherin expression led to the hypothesis that CSCs possess the capability of EMT [88].

Besides their multilineage developmental capacity and increased carcinogenesis potential [77], CSCs express a wide range of phenotypical epithelial, stromal, leukocytes, and vascular markers [75], as well as telomerase activity, which is considered the immortality gene [21]. Although endometrial carcinoma is associated with high telomerase activity [90], the mechanisms of causing exuberant cell proliferation during endometrial carcinogenesis are not fully known. Telomerase activity is also increased in normal endometrial cells due to specific hormonal influences. On the other hand, other concurrence factors

occur because of interactions of endometrial cells with cell populations not specific to the host environment that can influence telomerase and endometrial cell telomerase activity [90]. In this sense, understanding the mechanisms of regulating telomerase activity may lead to new treatment perspectives in endometrial pathology, involving stem cells [90].

However, the most probable mechanism considered to explain this phenomenon would be related to the relationship between the decreased postmenopausal estrogen level and telomerase activity deficiency, which cannot sustain the endometrial telomeres length and integrity, this leading to genetic instability and susceptibility of malignant transformation of improper proliferated epithelial cells [90].

By emphasizing the implication of telomerase activity in carcinogenesis and cell senescence processes, other studies highlighted the fact that inhibition of telomerase activity would be the target for a complementary therapy to existing chemotherapy [97]. Since telomerase activity has also been observed in other cell lines (germ cells, blood mononuclear cells, and fibroblasts), the potential effects of inhibition should be carefully evaluated, with consequences varying according to the pathway of the inhibition mechanism [97].

Endometrial stem cells application in regenerative medicine

ERCs potential has been tested in experimental studies, using *in vitro* and animal models and later on, as their safety has been demonstrated and clinical studies have been already performed or are being planned for the next future (Table 1).

Table 1 – *Endometrial stem cells application in regenerative medicine*

Application	Diseases	Current status	References
Uterine & endometrial diseases	Asherman syndrome	Clinical	[98]
Infertility	Sub-fertility due to thin endometrium	*In vitro* cultures of endometrial biopsies with hUCS	[99]
Bladder & pelvic prolapse	Pelvic prolapse Hernia Developmental & urinary bladder diseases	*In vitro* cultures of endometrial biopsies with different types of synthetic scaffolds	[61, 100–106]
Heart diseases	Ischemic heart diseases Heart failure	Clinical	[1, 2, 83, 107–112]
Musculoskeletal diseases	Muscular dystrophy	Clinical	[2, 109]
Pancreatic diseases	Diabetes mellitus	Experimental models	[2, 113, 114]
Hepatic diseases	Acute liver failure	Experimental model	[93, 115–117]
Dental diseases	Periodontal disease	Experimental model	[2, 65, 118]
Neurodegenerative diseases	Parkinson's disease Stroke Encephalitis	Experimental models	[2, 64, 78, 119–125]
	Multiple sclerosis	Clinical	[126]
Angiogenesis	Limb ischemia	Experimental model	[1]
Coagulation diseases	Thrombocytopenia	*In vitro* cultures	[2, 83]

hUCS: Human umbilical cord serum.

Preclinical and clinical safety of ERCs

The role of human ERCs in angiogenesis and its high level of immune privilege have been demonstrated in a murine competent hindlimb ischemia model [68].

The potential risk of uncontrolled ERC proliferation in recipients and genesis of endometriosis-like or fibroblast-type tumors has been infirmed, as no endometriosis has been developed after therapy with ERCs in mice experiments [127].

Considering the angiogenic effect of ERC, the concern that ERC could activate dormant tumors has been addressed by another experiment, which demonstrated an unexpected inhibitory effect on tumor growth [127].

ERCs in cell-based therapy of acquired endometrial diseases

Asherman syndrome is characterized by intrauterine fibrotic synechiae with the destruction of the endometrial basal layer, following miscarriage or curettage, being attributed to the destruction of endometrial stem cells [2]. Relatively recent, stem cell administration has been suggested as a possible therapy of this disease. Therefore, experiments on murine models of Asherman syndrome have been tested with successful results, using different types of stem cells measured by a higher pregnancy rate of treated animals attributed either to bone marrow-derived stem cells or either to trophic substances [128].

The application of an analogous procedure has been also tested in humans. The sources used during time in endometrial regeneration of this syndrome as providers of stem cells have been variable. The first use of autologous bone marrow-derived mesenchymal stromal cells in a patient with Asherman syndrome resulted in successful consecutive *in vitro* fertilization (IVF) [129]. Other sources have been also used, such as human amniotic mesenchymal stromal cells and autologous menstrual mesenchymal stromal cells [98, 130]. The use of menstrual MSCs resulted in increased endometrial thickness and the possibility of pregnancy (in two of seven cases) but limitations related to the sterility of the material and the purification methods remained to be further employed [98, 130].

ERCs in cell-based therapy of infertility

The endometrium quality is an important player in women fertility. An insufficiently thick endometrium may result in sub-fertility and failed IVF. Thus, the endometrial

stem cell therapy opens the perspective of endometrial regeneration and augmentation of fertility rates in the next future [131], analogous to the positive results already obtained in Asherman syndrome [129].

Endometrial MSCs initially tested in animal models [26] and later on in humans [129] have been used.

Furthermore, in order to accumulate all three types of cells involved in regeneration (endothelial progenitors, mesenchymal stem, and epithelial progenitors), endometrial biopsies obtained during picking up the ovum procedure and *in vitro* cultures with allogenic human serum from umbilical cord (hUCS) have been performed to avoid risk of pathogens and graft rejection [99].

ERCs in bladder tissue reconstruction and pelvic prolapse repair

The therapy of developmental and acquired urinary bladder diseases have been addressed by cell-based therapy, using BM-MSCs, endothelial progenitors [100], and, more recently, endometrial stem cells [100], on a nanofibrous scaffold [132]. The stimulation has been performed *in vitro* by EGF and keratinocyte growth factor (KGF) and lead to urothelial genotype and phenotype expression (positivity for CK20 and uroplakins) [63].

Similarly, in pelvic prolapse and hernia, different types of materials have been used, such as synthetic Vicryl® (polyglactin 910) meshes associated with bone marrow MSCs [133], mouse muscle-derived stem cells with porcine collagen of small intestine submucosa [101], and, lately, human endometrial MSCs from endometrial biopsy along with gelatin-coated polyamide scaffold meshes for fascia repair [102, 103]. These stem cells may differentiate into fibroblasts, induced by recombinant human connective tissue growth factor (CTGF) and into smooth muscle cells, induced by platelet-derived growth factor subunit B (PDGF-B) recruitment of vascular smooth muscle cells and pericytes, along with TGF-β1, which has an important paracrine effect [103, 104]. Moreover, microRNA-145 can assist the conversion of fibroblasts into smooth muscle cells [105].

Another application of endometrial stem cells expressing W5C5/SUSD2 in tissue engineering, using synthetic gelatin-coated polyamide (PA-G), as knit meshes [103], has been demonstrated in a rat skin injury model [106], followed by collagen deposition and angiogenesis. Although the endometrial stem cells have become undetectable around the meshes after two weeks from the subcutaneous implantation, an anti-inflammatory M2 population dominance compared to proinflammatory M1 population has been noticed at day 30, demonstrating immunomodulatory effect and potential use for pelvic prolapsed organs repair [134].

ERCs in cell-based therapy of myocardial infarction and heart failure

Ischemic heart disease may be treated with multipotent cells of variable origins, which are able to differentiate into cardiomyocytes [135]. Firstly, the potential of satellite cells has been tested [136], followed by autologous skeletal myoblasts [137], but limited by their lack of integration in myocardium and intrinsic arrhytmogenicity [138]. Another stage has been opened by bone marrow cells able to transdifferentiate into myogenic cells and to show angiogenic abilities [139]. Moreover, a progenitor cell population, SP cells, Lin-/c-kit+/Sca+ is able to differentiate into myogenic lineage, Lin-/c-kit+/CD34+/CD133+ [138]. These cells are able to repair cardiomyocytes histology, function and survival. This finding has initiated clinical studies, by using different cells as precursors, such as mononuclears [140], endothelial progenitor cells (EPCs) [141], bone marrow CD34+ cells [142, 143], MSCs [143, 144], inner cell mass of the blastocyst-origin embryonic stem cells [145], and induced pluripotent stem cells (iPSCs) generated from mature somatic cells [146]. Their administration has been intravenous or percutaneous transendocardial.

Allogenic mesenchymal precursor cells have been administrated intracoronary or percutaneous intramyocardial, forming stromal cells and multipotent cells which transdifferentiated into cardiomyocytes, possibly due to a paracrine effect [147]. This effect involves a cascade events, including cytoprotection of cardiomyocytes, endogenous stem cell recruitment, modulation of inflammation, angiogenesis stimulation, increased cardiac metabolism, improved contractility, and activation of host humoral activity [148], by downregulation of tumor necrosis factor (TNF) and caspase-1, overexpression of myocyte enhancer factor 2C (MEF2C) and GATA4, increased β-nicotinamide adenine dinucleotide, reduced (NADH), adenosine triphosphate (ATP), phosphorylated-glycogen synthase kinase (GSK)-3β, and phosphorylated-protein kinase B (Akt), and decreased phosphorylated-c-Jun *N*-terminal kinase (JNK) and oxidative stress [138].

The latest studies involved intracoronary injection of autologous cardiac stem cells [107], associated to a hyaluronan-gelatin hydrogel [149].

The limited benefit of transdifferentiation of these exogenous stem cells may be attributed to local signaling but this disadvantage may be overcome by the recruitment of endogenous cardiac stem and progenitor cells, which may be reprogrammed [150].

In order to retain the exogenous cells in myocardial site and to avoid their circulation in other organs, their association with different materials has been tested [138].

Intracoronary administration has been demonstrated to be superior compared to intramyocardial percutaneous administration, but combined delivery routes have been also tested [138].

The timing may be also important, and the administration at 5–30 days post-infarction may be beneficial according to some studies [151, 152] but denied by other studies [153, 154].

Although promising in animal experiments, the human benefits have been reduced probably due to origin and relative amount of implanted cells compared to the species size, inconsistent cell preparation methods, inappropriate timing of administration, type of intervention, delivery route, or limited number of selected patients [138].

Currently, the activation of endogenous cardiac stem cells is tested by using cell-based or cell-related gene therapy, such as locked nucleic acid-modified antisense miR-92a (*LNA-92a*) [155] and naked DNA plasmid encoding human SDF-1 (*JVS-100*) [155].

The clinical application of autologous myoblasts and autologous bone marrow cells has achieved significant results in heart function recovery [156, 157].

BM-MSCs have also demonstrated therapeutic effects in infarct patients and they have been used as "universal donor" cells, a single donor forming a bank of cells, which can be frozen and later on intravenously administrated [158].

MSCs are considered as immune privileged and immunomodulatory, showing optimal qualities for cardiac cell-based therapy, as they are poor stimulators of allogenic immunity and inhibitors of ongoing immune reactions [159]. Their administration in cardiac microenvironment leads to differentiation into cardiomyocytes, production of trophic factors, and angiogenesis stimulation [160].

The cell-based therapy has been tested in murine cardiac infarction models and the possibility of regeneration mechanism mediated by trophic factors produced by administrated cells, leading to stimulation of endogenous stem cells, has been considered [161].

ERCs have been successfully isolated and led to promising results in cardiac regeneration [2]. ERCs do not express fetal liver kinase-1 (Flk-1), CD14, CD34, or CD45 but express CD29, CD59, and GATA-4, the latter demonstrating cardiomyocytic progenitor cells qualities [2]. Furthermore, co-cultured menstrual-derived ERCs with murine cardiomyocytes express troponin I, connexin 43, α-actinin, along with pacemaker and action potential, thus demonstrating cardiomyocytic differentiation [2].

Moreover, ERCs ability of cardiac regeneration has proved to be superior to BM-MSCs in murine models [2]. Another study testing human endometrial stem cells in a rat model identified a panel of positive markers, such as CD29, CD90, CD105, and CD166, along with low positivity for c-kit, and negativity for CD34, CD45, and CD133 [108]. These cells have conferred preserved myocardium functionality when directly injected at the border of an infarcted area, the effect being more efficient in early post-infarction application of therapy [162].

Furthermore, the administration of ERCs post-infarct resulted in a better outcome, in terms of recovery of ejection fraction, reduction of fibrosis, and direct differentiation into cardiomyocytes when compared to MSCs [2].

Endometrial cells with regenerative abilities have been compared to BM-MSCs and a stronger expression of several components have been found, such as MMP-3, angiogenic cytokines (PDGF-BB and angiopoietin) [54, 83], and the stem cell potency-associated gene aldehyde dehydrogenase [83]. Moreover, ERCs possess a stronger expression of immunomodulatory genes, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), decay accelerating factor (DAF), pregnancy-associated glycoprotein 1, and neuronal pentatraxin, providing them an increased capacity to inhibit mixed lymphocyte reaction [83].

The first application of ERC therapy by intravenous administration in heart failure has been successful [108], avoiding the drawback of surgical invasiveness of trans-epicardially or transendocardially administration, or of a diminished amount of engrafting cells, risk of embolism and of ST segment elevations in anterograde administration *via* coronary artery [163].

Another technique has been developed in an analogous manner to the administration of oxygenated blood during coronary angioplasty by retrograde delivery into the coronary sinus [110], using post-capillary venules as entering site. Morphofunctional advantages consist in their smallest diameter, greatest transfer to interstitium, special biomechanical properties [1], expression of adhesion molecules involved in immune cells transfer, such as endothelial-leukocyte adhesion molecule 1 (ELAM-1) [164], CD18 [165], CD44 [166], and intercellular adhesion molecule-1 (ICAM-1) [167], also used for other types of stem/progenitors cells [168].

Cell therapy using retrograde administration has been already used in experimental animals [169] and subsequently in humans, leading to clinical improvement and reduction of the area of ischemic myocardium, according to single-photon emission computed tomography (SPECT) [170].

The application of ERC therapy using the retrograde administration in patients diagnosed with congestive heart failure, using escalating dose cohorts (from 50 to 200 million cells) resulted in promising preliminary data [1].

Endometrial stem cells produce a large panel of cytokines and growth factors in the myometrium, such as EGF, periostin, angiopoietin 1 (Ang1), PDGF, TGF-β2, nitric oxide, and VEGF, under ischemia stimulus [161]. These factors are considered as cardioprotective, possible mitogenic, pro-angiogenic, anti-apoptotic, stimulators of kinase pro-survival molecular pathways, initiators of cardiomyocyte return to cell cycle, and stimulation of the proliferative rate of both cardiomyocytes and endothelial cells [161].

It has been also speculated that the beneficial effect of stem cell therapy could be hampered by scar tissue and consequently an association with anti-collagen deposition therapy could improve future therapy based on stem cells [111, 169].

Considering that stem cells act *via* cytokines and growth factors survival kinase pathways [169], the tissue preservation and possible stimulation of endogenous regeneration may be the effect of their paracrine activity and therefore this finding supports the strategies of myocardial infarction therapy by using a certain cytokine profile [108, 171].

Endometrial stem cells have a different cytokine expression in culture, with increased amounts of EGF, TGF-β2, periostin, PDGF, and lack of VEGF in the absence of hypoxia, while the latter is mainly expressed by BM-MSCs [171].

Although stem cell therapy has demonstrated its validity, single cytokine therapy, using erythropoietin (Epo) [170] or granulocyte-colony stimulating factor (G-CSF) [172] has failed in clinical applications. This may suggest the necessity of association of multiple cytokines with possible complex interactions between them as more efficient, but this approach is limited either by expenses [171], either by difficulties in identifying the optimal secretome [172].

The early implementation of cell-based therapy is a requirement in order to limit infarct extension [173]. Furthermore, despite the unfavorable microenvironment for the transplanted stem cells due to ischemia, stimulation of AKT, extracellular signal-regulated kinase (ERK), and signal transducer and activator of transcription 3 (STAT3)

[174], along with p38 inhibition [169, 174] enhances their paracrine effect.

The use of autologous cells prevents early therapy and results in variation of cells quality and, thus, in therapeutic results. The use of endometrial stem cells may surpass all these limitations, as they proved to possess embryonic capacity and are easily obtained and cultured [161].

The main risk of immunorejection is largely prevented in MSCs due to their immunoprivileged status [161]. Moreover, the hypothesis of a secondary reaction after myocardial infarction regeneration, with inflammation neutralization due to endometrial stem cells immune modulatory abilities has been considered [68]. The risk of immunorejection may be also prevented by using HLA-typed endometrial stem cell lines [161].

ERCs in cell-based therapy of diabetes mellitus

The possibility of endometrial stem cells differentiation towards glucose-responsive insulin secreting cells represents an innovative approach in diabetes mellitus therapy [175] and may overcome the side effects of tissue rejection in islet-base transplantation and of teratomas development in autologous induced-pluripotent stem cells. As other MSCs (bone marrow, amnion or umbilical cord), endometrial stem cells are safer and may be reprogrammed to insulin-secreting cells [175]. Supplementary, they are easily available by endometrial biopsy or hysterectomy and they should be banked and matched to be available for transplantation [175].

In this respect, endometrial stem cells expressing CD90, CD246, and PDGFRβ have been directed in a stepwise experiment toward pancreatic differentiation [175]. The first step, consisted in one week incubation, in specific differentiation media, being characterized by expression of early developmental pancreatic genes, such as pancreatic and duodenal homeobox 1 (*PDX1*) and neurogenin 3 (*NGN3*) [2]. The following step aimed the cellular organization into an islet-like morphology in culture along with expression of mature beta cells markers, *e.g.*, glucose transporter 2 (GLUT2) and paired box 4 protein (PAX4) [2]. Supplementary, these cells are functionally active as they react to glucose *in vitro* and they react by insulin secretion in conditions of high concentrations of glucose [2]. These cells have been transplanted in diabetes mouse models, induced by streptozocin [175]. The results of transplant with pancreatic beta-like cells derived from endometrial stem cells has been promising, by diminishing the diabetes-associated complications, although without normalization of blood glucose levels [175].

Another approach has aimed to obtain spheroid bodies from endometrial MSCs composed of cells expressing mARN specific islet markers, such as GLUT2, NKX2, somatostatin, and glucagon, and glucose-dependent *in vitro* insulin production [113]. The transplant of these cells resulted in a better outcome compared to the previous type of experiment with glucose levels normalization, along with increased mice survival [2], opening perspectives in human therapy.

ERCs in cell-based therapy of musculoskeletal diseases

A large spectrum of traumatic and degenerative musculoskeletal diseases might be treated by addressing the cartilage regenerative capacity.

The first studies have used muscle precursor cells, allogenic myoblasts expressing dystrophin [176] demonstrating variable regenerative effects, only when associated with immune suppression and generally without significant improvement in muscular strength [109].

MSCs (CD90+/CD105+/CD73+/CD14-/CD34-/CD45-) are able to differentiate into tissues that have been previously injured due to chemokines, such as SDF-1 [177], CCL2 [178], and lysophosphatidic acid [179]. Furthermore, due to their intrinsic factor of protection against inflammatory damage for hematopoietic precursors, demonstrated in murine models [51], they may be more valuable. Additionally, the inhibition of chronic inflammations has been shown in models of autoimmune diseases and diabetes [180]. This process is achieved, in a milieu of active immune reaction, by indoleamine 2,3-deoxygenase [181], secretion of IL-10, leukemia inhibitory factor (LIF), and TGF-β [177], production of soluble human leukocyte antigen G (HLA-G) [182], along with express contact-dependent inhibitory molecules expression, *e.g.*, such as programmed death-ligand 1 (PD-L1) [109]. Moreover, paracrine factors are liberated by these cells, and activate endogenous stem cells proliferation and inhibit apoptosis.

However, there have been no reports of enhancement of contraction force in animal models [178]. Considered as superior due to enhanced levels of MMPs expression and powerful angiogenic activity, ERCs in association with CD34 umbilical cord blood cells, lymphocytes and placental matrix derived MSCs tested in a case of muscular dystrophy led to a functional improvement in the patient [109].

In order to amplify its natural self-repair ability, MSCs have been tested [2] and, furthermore, ERCs have been used by constructing polymer-based biomaterial support (poly-ε-caprolactone nanofibers), in chondrogenic differentiation media [2], its clinical validity waiting to be demonstrated by *in vivo* testing.

Furthermore, using a mouse model of Duchenne muscular dystrophy, with mutations of the *DMD* gene resulting in dystrophin glycoprotein anomalies and skeletal muscle degeneration [183], the regeneration capacity of stem cells derived from menstrual blood has been tested [2]. These cells, expressing mesenchymal cell markers, such as CD90, CD59, CD44, and CD29, have fused with thigh muscle myocytes, human dystrophin being detected by immunofluorescence in around 1.5% of muscle cells [180]. Moreover, in a co-culture assay, the differentiation of myogenin- and dystrophin-expressing myocytes has been demonstrated and, thus, the therapeutic value of this type of stem cells has been proved [180].

ERCs in acute liver failure

A less invasive procedure compared to liver transplant, without the limitations of available source, waiting lists, lack of donors, transplant rejection, and high cost, is that of cell transplantation [115], proven to have a higher survival rate in acute liver failure [115].

The source of hepatocytes has been variable, from embryonic stem cells up to bone marrow stem cells, mesenchymal cells, multipotent progenitor cells from umbilical cord, and adipocytes [115].

Recently, ERCs, along with hepatocyte progenitor-like (HPL) cells have been identified as the most potent in repairing acute liver failure, by an enhanced inhibition of inflammation post-transplantation [determined by down-regulation of IL-6 and cyclooxygenase-2 (COX-2) mRNA levels], amplified reparative activity, and increased capacity of regeneration in mice models, with improvement of liver functions [determined by tyrosine aminotransferase (TAT) and cholesterol 7-α-monooxygenase or cytochrome P450 7A1 (CYP7A1)] [116]. Regarding the mechanism of liver regeneration, two possible mechanisms may be proposed: either a migration of cells to the destroyed areas, followed by hepatocytes differentiation, either a paracrine trophic effect performed by cytokines and growth factors [93, 117].

ERCs in tooth regeneration

The regeneration of odontoblasts and parodontium has been the objects of intense research although numerous biomaterials have been already used in dental reconstruction.

During odontogenesis, a reciprocal induction process is taking place at the interface between internal epithelium of the enamel organ and ectomesenchymal cells of the dental papillae. This process is coordinated by BMPs, FGF, sonic hedgehog (SHH), and WNT, resulting in differentiation of ameloblasts and odontoblasts, respectively [184].

The secretory activity of ameloblasts is limited, as they are degenerating at the end of amelogenesis, while the secretory activity of odontoblasts is persisting during the post-eruptive tooth lifespan, during secondary dentin synthesis.

Odontoblasts regenerative ability has been demonstrated by research performed both in permanent and in deciduous dentition, supported by the identification of MSCs in dental pulp. These cells are able to differentiate, according to the type of stimuli, into ondontoblasts, chondroblasts, adipocytes, neurons, and possibly osteoblasts. Therefore, these cells may constitute the source for alveolar bone regeneration [118].

The origin of mesenchymal pulp stem cells is currently an intensely debated subject, literature suggesting as possible sources the bone marrow, dental pulp, or human exfoliated deciduous teeth [185]. Despite their therapeutic potential, the access to these pulp stem cells is not easy and, furthermore, evident limitations are attributed to their low number and their reduced capacity of regeneration.

Consequently, the idea of a possible involvement in bone tooth regeneration of CD146+/PDGFRβ+ [56] endometrial stem cells has been launched, considering their large capacity of differentiation, including the osteoblastic line [55]. These endometrial stem cells are easily accessible and their rate of proliferation is much higher when compared to pulp or bone marrow stem cells (1.25 *vs.* 0.1–0.01%) [24]. The molecular phenotype of these cells has revealed expression of CD105, CD90, along with their lack of expression of CD31 and CD34 [54, 55, 65], showing *in vitro* ability to differentiate into adipocyte and osteocyte lines [65]. Additionally, other studies have revealed their possibility to differentiate into other cellular lines [2, 67], making endometrial stem cells potent candidates to bone tooth regeneration. Although there are limited results of their exploitation in dentine regeneration, current investigations open new promising perspectives.

However, osteoblastic potential of endometrial stem cells and their capacity of dentine regeneration are intimately associated to the complex microenvironment of dental pulp and therefore its modulation could represent the mean for future therapies for alveolar bone regeneration.

ERCs in megakaryocyte and platelets production

The huge potential of ERCs has been also exploited by their capacity to differentiate into megakaryocyte-like platelet producing cells [83]. This has been tested in thrombopoietin-supplemented culture, being demonstrated by CD41a and CD42b expression. Supplementary, these cells expressed the ability to generate cells with all the ultrastructural features known in circulating platelets and functionally could bind fibrinogen *in vivo* under thrombin stimulus [83]. Further clinical applicability should be tested in the next future [2].

ERCs in angiogenesis

Given the intrinsic capacity to activate angiogenesis, by expressing high levels of vascular growth factors, such as VEGF, EGF, PDGF, and MMPs, and the ability to stimulate *in vitro* proliferation of umbilical vein endothelium [1, 54], ERCs have been tested in a mouse model of limb ischemia with successful clinical utility. Concomitantly, the experiment also demonstrated the immune privileged status of ERCs, confirmed also in mixed lymphocyte reactions, supported by the ability to stimulate IL-4, with immunosuppressive function, and to decrease lymphocytes proliferation and the expression of TNF and interferon gamma (IFNγ) [68].

ERCs in neural regeneration – a challenge for the future

The observations that ischemia stimulates endogenous neural cells to proliferate resulted in different attempts to enhance endogenous neurogenesis (by anti-inflammatory drugs, growth factors, nitric oxide, substance-P, galectin-1 or by immortalized neural stem cells exogenous transplantation [119] but their clinical application being limited by reduced availability of donor cells [186].

Although embryonic cells exhibit extensive pluripotentiality, by promoting transdifferentiation into neuronal cells, their use is hampered by difficult proliferative regulation and possible teratogenesis [186].

It seems that, in stroke, stem cells promote an angiogenic effect, *via* several growth factors, such as VEGF, IGF-1, and FGF-2 or possibly *via* a vessel-guided neuronal migration mechanism [187]. The finding that endothelial progenitor cells possess neural repairing abilities led to the association of this type of cells in order to amplify the effects of neural stem cells [188]. Although

previously thought that cell migration to the stroke site is the main critical event, it has been demonstrated that, even when not detected, some types of stem cells are initiators of repair, probably due to their paracrine effect [189]. Angiogenic agents, along with neurotrophic factors are promising for therapy, requiring more studies regarding their long-term effectiveness and stability [186].

In vitro stroke models have demonstrated that menstrual blood-derived endometrial cells are showing the ability to produce trophic factors, such as brain-derived neurotrophic factor (BDNF), neutrotrophin-3 (NT3), and VEGF [55, 189] and the rates of cells survival is increased by co-culturing with rat primary neurons [189]. These cells cultured in specific media display proliferative and clonogenic abilities, express microtubule associated protein 2 (MAP2) and nestin [55, 189], without tumorigenic potential [54]. The observation that endometrial cells are not differentiating *in vivo* and retain OCT4 expression led to the suggestion that their neuroprotective action might be the result of secretory factors of the endometrial stem exosome [189]. Furthermore, their migration to injured as well as non-injured areas, without evidence of differentiation, is suggesting another mechanism of action that needs future clarification [120].

Adult endometrial stromal stem cells are able to differentiate into neurons that produce dopamine and therefore they have been used in a Parkinson's disease animal model [121]. This has been the first *in vitro* differentiation to neuron-like cells expressing tyrosine hydroxylase (TH) and nestin derived from endometrial derived stem cells expressing PDGFRβ, CD90, and CD146 [121]. In addition to TH, the enzyme responsible for dopamine production regulation, barium-sensitive inward rectifier potassium channels have been identified by electrophysiological measurements, as specific for the central nervous system [121].

Dopamine-producing cells have been regenerated in a mouse model of Parkinson's disease induced with a neurotoxin, namely 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [122]. Following the transplantation of endometrial derived stem cells into the striatum, a migration towards substantia nigra has been observed, along with morphology changes to a neuron type associated with high levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), as a dopamine metabolite [122]. These effects may be considered as an association of *in vivo* differentiation into dopamine-producing cells and prevention of endogenous neural death [121]. The same experiment in primates' models, using allogenic endometrial-derived stem cells has demonstrated a wider effect in the transplanted side of the brain, involving a more complex mechanism than that seen in mice models [190]. These models open new direction for Parkinson's disease therapy development.

Another potential approach in neural therapy is the possibility to obtain cholinergic neuron-like cells *in vitro*, by stimulation of CD146-positive endometrial-derived stem cells with bFGF and nerve growth factor (NGF) [64]. The cells obtained by this procedure showed a neuronal-type morphology and expressed MAP2, neurofilament L, and choline acetyltransferase, their further characterization still awaiting [64].

The possibility to regenerate oligodendrocytes lost during inflammatory diseases associated with demyelinization, *e.g.*, multiple sclerosis, are promising in brain and spinal cord disorders therapy [2]. *In vitro*, endometrial MSCs expressing CD44, CD90, CD105, and CD146, in bFGF, EGF, PDGF-AA, and thyroid hormone conditioned medium may achieve an oligodendrocyte phenotype [191]. These cells show a bipolar morphology and express a panel of mRNA and/or immunopositivity of oligodendrocyte markers, such as oligodendrocyte transcription factor (OLIG2), SOX10, PDGFRα, O4, and A2B5 [123].

Supplementary to oligodendrocytes regeneration by neural stem cells, embryonic stem cells, human olfactory epithelial cells, human fetal MSCs, induced pluripotent stem cells, and bone marrow stem cells, endometrial stem cells have been proposed as new means for specific therapy [124]. Among the factors that are regulating oligodendrocyte differentiation, miR-219 is promoting the process by inhibition of PDGFRα, Hes5, forkhead box J3 (FoxJ3), Sox6, and ZFP238, followed by PDGF-AA inhibition of proliferation and initiation of oligodendrocytes differentiation [123].

Furthermore, due to immunomodulatory effect, the therapy with endometrial derived stem cells may improve the outcome of encephalitis [2]. In mice models, endometrial-derived stem cells expressing HLA-BC, SH4, CD29, CD73, and CD90 reduced the central nervous system activity of proinflammatory Th17- and Th1-infiltrating cells and increased splenic regulatory T-cells anti-inflammatory panel of cytokines, such as indoleamine 2,3-dioxygenase, Foxp3, IL-10, and IL-27 [192].

The perspective of application of stem cell therapy in stroke may stimulate angiogenesis [186, 187] and may reduce neural death, being so far tested in rat models [189]. Intracerebrally injected ERCs expressing CD117 lead to better neuronal survival, better functional outcome, although resulted in similar functional outcomes compared to intravenous administration route, in experimental models [189]. Apparently, the effect is mediated by NT3, BDNF [120], and VEGF [191] as neurotrophic factors and reduced neural death induced by hypoxia, as demonstrated by *in vitro* cultures [189].

The first use of ERCs in a clinical trial has been dated in 2008, involving patients with multiple sclerosis, which have received intravenous and intrathecal injections, without any adverse events reported in their four-year follow-up and without any immune reaction or tumor development [126]. However, further development would demonstrate their real value in therapy.

Final remarks

Recent progresses in endometrial stem cells research made in the last decade may generate new hypotheses regarding eutopic regeneration and ectopic implantation.

The facility in obtaining stem cells from endometrium and their high proliferative ability make them ideal candidates for cell-based therapies. The identification of new markers of endometrial stem cells is necessary in order to facilitate their isolation and promising applications.

Currently, beside uterine-acquired diseases and infertility, endometrial stem cells have been tested in a large

spectrum of clinical applications. Although their application is yet limited, more clinical trials are necessary to surpass these limits, to improve, and to extend the spectrum of endometrial stem cells exploitation.

The perspectives of development of an endometrial stem cells bank with a large spectrum of HLA-typed cell lines may prevent immunorejection, as the main risk of their application.

In order to ameliorate drawbacks of stem cells and to enhance their synergistic activity, a useful approach is their combination resulting in an enhanced trophic, anti-inflammatory, and angiogenic effect.

The great potential of endometrial cells for cell therapies arise from their completely xeno-free derivation, allogenic use, possibility of large-scale therapeutic doses production, safety, reproducibility, and chance to overcome the drawbacks associated with autologous therapies.

In order to overcome hostile environment of an injured tissue, the association of endometrial stem cells with other stem cells, possibly with added medium, in specific cases opens the perspective of specific combination available as standardized therapeutic means in the next future.

Conflict of interests

The authors declare that they have no conflict of interests.

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